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Comparative morphometry of the nasal cavity in rats and mice

E. A. GROSS, J. A. SWENBERG, S. FIELDS AND J. A. POPP

Department of Pathology, Chemical Industry Institute of Toxicology, P.O. Box 12137, *Research Triangle Park, NC* 27709, *U.S.A.*

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INTRODUCTION

As the first part of the respiratory system in mammals, the nasal cavity and mucosa warms, moistens, and filters incoming air and houses the receptors for smell (Sorekin, 1977). The surface of the nasal cavity is comprised of squamous epithelium, respiratory epithelium, and olfactory epithelium with distinct lines of demarcation between epithelial types. Published reports dealing with various morphological parameters of the nasal cavity are incomplete. Adams (1972) has reviewed and tabulated the anatomical information available. Variability in animal ages and weights, incomplete information concerning strains used, measurement of only single parameters and extreme variability in methods make interpretation and comparison of published data difficult.

Morphometric analysis of the rodent nasal cavity has been undertaken by our laboratory to determine systematically epithelial surface area and nasal cavity volume. The specific objectives of this study were (1) to determine total nasal cavity surface area and volume of F-344 rats and B6C3F1 mice; (2) to quantify the surface areas of the squamous, respiratory and olfactory epithelia in these same rats and mice; (3) to determine the variation in surface area and volume within a single species; (4) to compare the various morphological parameters between the two species studied.

MATERIALS AND METHODS

Animals were obtained from Charles River Breeding Laboratories (Wilmington, Ma), housed in plastic cages with heat-treated hardwood chip bedding, exposed to a light–dark cycle of 12 hours, and given food (Wayne Certified Lab Blocks, Allied Mills, Chicago, Ill.) and water *ad libitum*. Two groups of three male CDF® (F-344)/CrlBr (Fischer-344) rats aged 7 and 16 weeks (116 and 288 g) and two groups of three male B6C3F1/CrlBr mice, also aged 7 and 16 weeks (30 and 33 g) were used to study various morphological parameters of the nasal cavity. These species were chosen because they are so commonly used in experimental research. All animals were anaesthetized with methoxyflurane (Pitman-Moore, Inc.) and then killed by vascular perfusion through the heart with 6% dextran in physiological saline (Cutter Laboratories, Inc., Berkeley, Ca). Fixation was achieved by vascular perfusion through the heart with 10% neutral buffered formalin for 8 minutes, using a 43 inch fluid column and an 18 G gavage needle. The heads were skinned and the lower jaws removed. Heads were removed and placed in a 10% neutral buffered formalin solution for 24 hours. The skull bones were decalcified in Cal-Ex (Fisher Scientific Co., Fairlawn, NJ) for 2–3 days. For the rats, the heads were cut in half transversely just anterior to the molar teeth and both blocks of tissue were embedded in paraffin.

For the mice, each head was embedded in a single block. The blocks were oriented to provide transverse sections when cut on a Leitz microtome. Sections of 10 μm were collected every 500 μm through both blocks from each rat, while 5 μm sections every 200 μm were collected from each mouse block. Sections were stained with haematoxylin and eosin. For morphometric analysis, each section was photographed on a Zeiss Tessovar photomacrographic system (Carl Zeiss Inc., New York, NY) and printed at a final magnification of $\times 14$ to $\times 34$ with magnification held constant for all sections of any one animal. Using light microscopy, the nasal epithelium was classified as squamous, respiratory or olfactory according to the definitions of Weiss & Greep (1977). The nasal epithelium of each photograph was colour coded for later analysis on a Zeiss Videoplan computerized image analysis system (Carl Zeiss Inc., New York, NY). With the final print magnification of each animal set at the beginning of analysis, the measuring program (version 4·61) was used for all measurements. The parameters selected for use from the program were length, area, and perimeter. Length was defined as the distance along the perimeter of the nasal cavity of each segment of a particular type of epithelium. Area was the area of each cross section of the nasal cavity. Perimeter was the length of epithelium of all types found in each cross section. This provided a check for each section analysed because the total of all the squamous, respiratory and olfactory epithelium lengths should be the same as the separate perimeter measurement.

For calculating nasal cavity volume and surface area from the obtained area and perimeter measurements of sections, each rat section was considered to project 250 μm anterior and 250 μm posterior to its original location in the snout (sections were 500 μm apart), except for the first and last sections of each block which were not projected outside the limits of actual collected tissue. Each mouse section was projected 100 μm anterior and 100 μm posterior to its location, and again there was no projection outside actual tissue blocks. With the exception of the septal olfactory organ (Organ of Rodolfo-Massera) (Rodolfo-Massera, 1943) and the nasopharynx, the character of the nasal epithelium from anterior to posterior changes from squamous epithelium to respiratory epithelium to olfactory epithelium. Areas of transition were split equally between the two epithelial types observed. Islands of respiratory epithelium were observed in the olfactory region. Length calculations were defined as [(number of slides within the described limits -2) $\times$ 500 μm] + [2 $\times$ 550 μm] for rats, and [(number of slides) $\times$ 200 μm] for mice.

Nasal cavity volume was calculated as the sum of the volumes represented by each section. This volume was calculated as surface area of the nasal cavity for each section times the distance that section was projected in the nose (250 μm for block ends, 550 μm for block beginnings and 500 μm for all other sections in rats and 100, 250 and 200 μm for mice). Total nasal surface area was calculated as the sum of the surface length (perimeter or length) of each epithelial type in each tissue section times distance as used in the volume calculations. Where applicable, means and standard deviations were calculated.

For the rats, evaluation of the volume and surface area of the nasal cavity began between 2000 and 2500 μm anterior to the posterior surface of the incisor teeth at the point of eruption through the gum. This point was approximately at the level of the nasal apex (Adams, 1972). Evaluation of the nasal epithelium extended to between 0 and 500 μm past the posterior limit of the ethmoid turbinate. Selection of these limits ensured that only sections with a complete perimeter were used for volume calculations and that the entire nasal cavity was evaluated. For the mice, evaluation

Table 1. *Nasal cavity: morphometric data*

	Rat		Mouse	
	7 weeks (115 g)	16 weeks (288 g)	7 weeks (30 g)	16 weeks (33 g)
Length (mm)	7·3 ± 0·0*	9·1 ± 0·3	4·9 ± 0·7	5·1 ± 0·08
Volume (mm^3)	155·5 ± 1·3	256·7 ± 4·1	32·5 ± 3·2	31·5 ± 2·1
Surface area (mm^2)				
Squamous epithelium	27·7 ± 1·9	44·2 ± 5·2	20·9 ± 0·4	20·6 ± 2·2
Respiratory epithelium	352·4 ± 4·9	623·1 ± 14·0	132·4 ± 5·7	133·9 ± 4·6
Olfactory epithelium	418·5 ± 19·2	675·2 ± 43·0	125·5 ± 4·0	136·9 ± 7·3
Total surface area	798·6 ± 55·0	1343·5 ± 55·0	277·7 ± 16·1	289·0 ± 13·1

* Values are expressed as mean of 3 animals ± standard deviation.

Table 2. *Nasal surface area: percentage composition of various epithelial types*

	Rat		Mouse	
	7 weeks	16 weeks	7 weeks	16 weeks
Squamous epithelium	4·0 %	3·0 %	7·5 %	7·0 %
Respiratory epithelium	44·0 %	47·0 %	47·5 %	46·0 %
Olfactory epithelium	52·0 %	50·0 %	45·0 %	47·0 %

of the nasal cavity for volume and surface area determination began between 2000 and 2500 μm anterior to the posterior surface of the incisor tooth at the point of eruption through the gum and extended to between 0 and 200 μm past the posterior limit of the ethmoid turbinates.

RESULTS

Rats

Nasal lengths of the two different age groups were determined utilizing subgross markers identified on the photographs of the sections. The anterior point was defined as the posterior surface of the incisor tooth at the point of eruption through the gum. The posterior point was defined as the most anterior section with an incomplete septum. These lengths were 7·3 mm in 7 weeks old rats and 9 mm in 16 weeks old rats, a 20 % increase. The surface area of the nasal cavity in rats increased between 7 and 16 weeks from 798·6 to 1343·5 mm^2 (Table 1).

The surface area of the nasal cavity that is comprised of each epithelial type is presented in Table 1. The percentage composition data are shown in Table 2. While the total nasal surface area increased by 68 % between 7 and 16 weeks, squamous and respiratory epithelial surfaces increased by 60 and 77 %, respectively. The surface area of the olfactory epithelium increased by 61 %. The septal olfactory organ measured 0·47 ± 0·20 mm^2 in 7 weeks old rats and 1·33 ± 0·05 mm^2 in 16 weeks old rats. The ratio of nasal surface area (in mm^2) to body weight (in grams) decreased from 6·94:1 to 4·60:1 between 7 and 16 weeks. The ratio of nasal surface area (in mm^2) to nasal volume (in mm^3) increased only slightly, from 5·1:1 to 5·2:1, over that same time.

Mice

Nasal lengths of the two different age groups were determined utilizing the same subgross markers used in the rats and were 4·9 mm in 7 weeks old mice and 5·1 mm in 16 weeks old mice. The nasal volume data are presented in Table 1. No significant change in nasal volume of the mouse was noted between 7 and 16 weeks. With the exception of the septal olfactory organ and the nasal pharynx, the nasal epithelium changed smoothly from squamous epithelium to respiratory epithelium to olfactory epithelium (anterior to posterior). The surface area of the nasal cavity that was represented by each epithelial type is shown in Table 1. The percentage composition data are shown in Table 2. Between 7 and 16 weeks there was no significant change in either the total surface area of the nasal epithelium or in the percentage composition of squamous, respiratory or olfactory epithelia. The septal olfactory organ in mice was the same size ($\sim$ 0·28 mm^2) at 7 and 16 weeks.

DISCUSSION

An examination of published reports on nasal volume and epithelial surface area reveals that normal morphologic and morphometric characteristics have not been thoroughly examined. Adams (1972) tabulated the results of ten authors reporting on nine different genera. Several of these references reported on only one or two animals and most described only a single morphometric parameter. Only three of these authors (Dieulafe, 1906; Gurtovia, 1966; Adams, 1972) dealt with rodents.

The present study provides basic morphometric information pertaining to two rodent species commonly used in biomedical research and ratios of the various morphometric parameters which are of particular interest in anatomical and respiratory physiological research. The total surface areas of the nasal cavity in Fischer-344 rats at 7 and 16 weeks were 799 and 1344 mm^2, respectively. The total surface areas of the nasal cavity in B6C3F1 mice at 7 and 16 weeks were 278 and 289 mm^2, respectively. The ratios of nasal surface area (mm^2) to body weight (grams) were 6·9:1 in 7 weeks old and 4·6:1 in 16 weeks old rats, respectively. This shows that the rat nasal cavity surface is increasing in size at a slower rate than the animal's body weight. The ratio of nasal surface area to body weight of B6C3F1 mice is essentially the same at 7 and 16 weeks (9·2:1 and 8·2:1). The ratio is greater in mice than in rats, showing that while rats have a greater surface area of nasal epithelium in absolute terms, mice have a greater surface area relative to body weight. Unlike the rat, the nasal cavity surface of mice is increasing in size at approximately the same rate as body weight.

Adams (1972) presented a ratio of total nasal surface area to metabolic body size where metabolic body size is defined as weight to the 3/4ths power (Guyton, 1947*a*, *b*). For the B6C3F1 mouse the ratios of nasal surface area to metabolic body size were 24·2:1 and 22·5:1 for 7 and 16 weeks old mice, respectively. These values are slightly higher than published values for the mouse *Peromyscus maniculatus* which has a smaller metabolic body size (Adams, 1972) than the B6C3F1 mouse. By comparison, the ratios of nasal surface area to metabolic body size in the Fischer-344 rat are slightly smaller (22·6:1 and 19·2:1) than those of the B6C3F1 mouse at both ages.

The percentage of the nasal surface area covered by the various epithelial types lining the nasal cavity (Table 2) is similar in both species and at both ages examined. The ratios of non-olfactory epithelium to olfactory epithelium are 0·91:1 and 0·99:1

for 7 and 16 weeks old rats. These values are slightly smaller than those reported by Dieulafe (1906); however, he did not give the weight or strain of rat used. The ratios of non-olfactory epithelium to olfactory epithelium are 1·22:1 and 1·13:1 for 7 and 16 weeks old mice, respectively and their constancy supports the suggestion of Adams (1972) that a definite surface area of non-olfactory epithelium is required to warm and humidify air for a given area of olfactory epithelium. Thus the ratio of non-olfactory to olfactory epithelium is constant for an individual species regardless of age, body weight, or sex.

The septal olfactory organ of Fischer-344 rats measured $0{\cdot}47 \pm 0{\cdot}2$ mm^2 at 7 weeks and $1{\cdot}32 \pm 0{\cdot}05$ mm^2 at 16 weeks. This was in agreement with the published reports of Bojsen-Møller (1975) and Rodolfo-Masera (1943). In the present study, no difference was observed in the size of the septal olfactory organ in 7 and 16 weeks old mice. The location, size, and shape of the septal olfactory organ in B6C3F1 mice is in agreement with published reports on *Peromyscus* (Adams & McFarland, 1971).

The volumes of the nasal cavity of 7 and 16 weeks old rats were 155·5 and 256·7 mm^3, respectively. In mice, this volume is 32 mm^3 at both ages studied. The nasal surface area to nasal volume ratio remains essentially the same for both ages of rats (5·1 and 5·2) and mice (8·5 and 9·2), although it is greater in mice. This ratio, coupled with the epithelial type composition data, shows that the mouse has more respiratory epithelium available for filtering air per unit volume of the nasal cavity than the rat.

This study provides basic anatomical information on the nasal cavity of two rodents with widespread scientific use. The data have application in anatomical and respiratory physiological research. Such base-line data can also be used in toxicology to help quantify the 'dose' of inhaled chemical reaching the individual epithelial types and will be useful in understanding species differences in response to the same concentration of inhaled chemical. For example, in inhalation experiments with formaldehyde, Barrow, Steinhagen & Chang (1981) utilized nasal morphometric data and minute volumes for rats and mice to calculate the 'dose' of formaldehyde available for absorption by the nasal mucosa. According to their calculations, during 10 minute exposures to 15 ppm formaldehyde, the nasal mucosa of the mouse is exposed to half the amount of formaldehyde per unit area as is that of the rat. This dose correlates well with tumour data, where the incidence of nasal carcinoma is similar in rats exposed to 6 ppm and mice exposed to 15 ppm of formaldehyde vapour (Kerns, Donofrio & Pavkov, 1980). This methodology could also be used to quantify changes in epithelial types (e.g. squamous metaplasia) occurring in subchronic and chronic inhalation studies.

SUMMARY

The distribution of the various epithelial types lining the nasal cavity in normal 7 and 16 weeks old male Fischer-344 rats and male B6C3F1 mice has been mapped at the light microscopic level. Photographs of transverse sections of the nose were analysed using a Zeiss Videoplan computerized image analysis system programmed for measurement and evaluation of count, area, perimeter and length.

In rats, the volumes of the nasal cavity at 7 and 16 weeks are 156 and 257 mm^3 respectively; while in mice the nasal cavity volume is essentially the same (32·5 and 31·5 mm^3) at the same two ages.

Total surface areas of the nasal cavity in rats at 7 and 16 weeks are 799 and 1344 mm^2 respectively; and in mice 278 and 289 mm^2. The percentages of the nasal

cavity surface lined by squamous, respiratory and olfactory epithelium are similar at both ages in both species. Applications and significance of these data are discussed.

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